

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 08 March 2001 (08.03.01)	
International application No. PCT/DK00/00350	Applicant's or agent's file reference P199900792WO
International filing date (day/month/year) 29 June 2000 (29.06.00)	Priority date (day/month/year) 29 June 1999 (29.06.99)
Applicant BLIRUP-JENSEN, Søren et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:18 January 2001 (18.01.01)☐ in a notice effecting later election filed with the International Bureau on:2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer C. Cupello
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PARENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

HOFMAN-BANG ZACCO A/S
Hans Bekkevolds Allé 7
DK-2900 Hellerup
DANEMARK

Date of mailing (day/month/year) 03 September 2001 (03.09.01)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference P199900792WO	
International application No. PCT/DK00/00350	International filing date (day/month/year) 29 June 2000 (29.06.00)

1. The following indications appeared on record concerning:		
<input type="checkbox"/> the applicant	<input type="checkbox"/> the inventor	<input checked="" type="checkbox"/> the agent
<input type="checkbox"/> the common representative		
Name and Address HOFMAN-BANG A/S Hans Bekkevolds Allé 7 DK-2900 Hellerup Denmark	State of Nationality	State of Residence
	Telephone No. 45 39 48 80 00	
	Facsimile No. 45 39 48 80 80	
	Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:		
<input type="checkbox"/> the person	<input checked="" type="checkbox"/> the name	<input type="checkbox"/> the address
<input type="checkbox"/> the nationality		
<input type="checkbox"/> the residence		
Name and Address HOFMAN-BANG ZACCO A/S Hans Bekkevolds Allé 7 DK-2900 Hellerup Denmark	State of Nationality	State of Residence
	Telephone No. 45 39 48 80 00	
	Facsimile No. 45 39 48 80 80	
	Teleprinter No.	
3. Further observations, if necessary:		
4. A copy of this notification has been sent to:		
<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned	
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned	
<input checked="" type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:	

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Céline Faust
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference P199900792W0	FOR FURTHER ACTION		see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/DK 00/ 00350	International filing date (day/month/year) 29/06/2000	(Earliest) Priority Date (day/month/year) 29/06/1999	
Applicant DAKO A/S			

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

BENZOATE BUFFERS FOR ZONE ELECTROPHORESIS AND IMMUNOFIXATION

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 00/00350

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N33/561 G01N33/48 G01N33/68 G01N27/447 B01D57/02
G01N33/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TARNAI, M. ET AL: "Capillary electrophoretic separation of mono- and dimaltosyl-.beta.-cyclodextrins and determination of the stability constants of their benzoate complexes." CHROMATOGRAPHIA, vol. 48, no. 5-6, 1998, pages 383-387, XP000856531 cited in the application page 384, column 1, paragraph 4; figures 1-4 --- -/--	1-22



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

14 November 2000

Date of mailing of the international search report

24/11/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
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Authorized officer

Hart-Davis, J

INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 00/00350

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>TAWALI, A. B. ET AL: "Determination of phytic acid content of soya beans during tempeh production using capillary electrophoresis."</p> <p>DEUTSCHE LEBENSMITTEL-RUNDSCHAU, vol. 94, no. 1, 1998, pages 28-30, XP000856532</p> <p>cited in the application</p> <p>page 28, column 2</p> <p>---</p>	1-22
X	<p>AHUMADA, I. ET AL: "Determination of organic acids and phosphate in soil aqueous extracts by capillary zone electrophoresis."</p> <p>COMMUNICATIONS IN SOIL SCIENCE AND PLANT ANALYSIS, vol. 30, no. 1-2, 1999, pages 213-220, XP000856533</p> <p>cited in the application</p> <p>abstract</p> <p>---</p>	1-22
X	<p>ARELLANO, M. ET AL: "Capillary electrophoresis and indirect UV detection as a fast and simple analytical tool for bacterial taxonomy."</p> <p>J. CHROMATOGR., A (PRESENTED AT THE 9TH INTERNATIONAL SYMPOSIUM ON HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS HELD IN ANAHEIM, CA, USA, 26-30 JAN 1997), vol. 781, no. 1-2, 1997, pages 497-501, XP004094582</p> <p>cited in the application</p> <p>figure 2</p> <p>---</p>	1-22
X	<p>LUNA, E. A. ET AL: "Evaluation of the utility of capillary electrophoresis for the analysis of sulfobutyl ether.beta.-cyclodextrin mixtures"</p> <p>JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS, vol. 15, no. 1, 1996, pages 63-71, XP000856460</p> <p>cited in the application</p> <p>abstract</p> <p>---</p>	1-22
X	<p>CHANKVETADZE, BEZHAN ET AL: "Capillary electrophoresis enantioseparation of noncharged and anionic chiral compounds using anionic cyclodextrin derivatives as chiral selectors"</p> <p>JOURNAL OF CAPILLARY ELECTROPHORESIS, vol. 2, no. 5, 1995, pages 235-240, XP000856503</p> <p>cited in the application</p> <p>abstract; figures 1,2</p> <p>---</p> <p style="text-align: center;">-/--</p>	1-22

INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 00/00350

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 891 422 A (CARLIN EDWARD ET AL) 6 April 1999 (1999-04-06) column 8, line 36 - line 36; claim 1 ----	1-10,22
A	US 4 321 119 A (AMBLER JEFFREY) 23 March 1982 (1982-03-23) cited in the application the whole document -----	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/DK 00/00350

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5891422	A	06-04-1999	NONE	
US 4321119	A	23-03-1982	AU 6189780 A	14-05-1981
			EP 0034233 A	26-08-1981
			JP 56076040 A	23-06-1981

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REC'D 23 JUL 2001

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

161

Applicant's or agent's file reference P199900792 WO	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/DK00/00350	International filing date (day/month/year) 29/06/2000	Priority date (day/month/year) 29/06/1999
International Patent Classification (IPC) or national classification and IPC G01N33/561		
Applicant DAKO A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 7 sheets, including this cover sheet.

- ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 18/01/2001	Date of completion of this report 19.07.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Thiele, U Telephone No. +49 89 2399 8643 

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK00/00350

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):
Description, pages:

1-20 as originally filed

Claims, No.:

1-22 with telefax of 28/06/2001

Drawings, sheets:

1/5-5/5 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK00/00350

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	9,11-20
	No:	Claims	1-8,10,21-22
Inventive step (IS)	Yes:	Claims	11-20
	No:	Claims	1-10,21-22
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Section V

- 1) Reference is made to the following documents:

D1: US-A-5 891 422

D2: TARNAI, M. ET AL: 'Capillary electrophoretic separation of mono- and dimaltosyl-.beta.-cyclodextrins and determination of the stability constants of their benzoate complexes.' CHROMATOGRAPHIA, vol. 48, no. 5-6, 1998, pages 383-387, cited in the application

D3: TAWALI, A. B. ET AL: 'Determination of phytic acid content of soya beans during tempeh production using capillary electrophoresis.' DEUTSCHE LEBENSMITTEL-RUNDSCHAU, vol. 94, no. 1, 1998, pages 28-30, cited in the application

D4: AHUMADA, I. ET AL: 'Determination of organic acids and phosphate in soil aqueous extracts by capillary zone electrophoresis.' COMMUNICATIONS IN SOIL SCIENCE AND PLANT ANALYSIS, vol. 30, no. 1-2, 1999, pages 213- 220, cited in the application

D5: ARELLANO, M. ET AL: 'Capillary electrophoresis and indirect UV detection as a fast and simple analytical tool for bacterial taxonomy.' J. CHROMATOGR., A (PRESENTED AT THE 9TH INTERNATIONAL SYMPOSIUM ON HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS HELD IN ANAHEIM, CA, USA, 26-30 JAN 1997), vol. 781, no. 1-2, 1997, pages 497-501, cited in the application

D6: LUNA, E. A. ET AL: 'Evaluation of the utility of capillary electrophoresis for the analysis of sulfobutyl ether.beta.-cyclodextrin mixtures' JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS, vol. 15, no. 1, 1996, pages 63-71, cited in the application

D7: CHANKVETADZE, BEZHAN ET AL: 'Capillary electrophoresis enantioseparation of noncharged and anionic chiral compounds using anionic cyclodextrin derivatives as chiral selectors' JOURNAL OF CAPILLARY ELECTROPHORESIS, vol. 2, no. 5, 1995, pages 235-240, cited in the application

- 2) Introductory remarks:

The term "Zone electrophoresis and/or immunofixation buffer" is identical in scope

with "Buffer for use in zone electrophoresis and/or immunofixation" (see claim 1).

A term such as "for [use] in zone electrophoresis and/or immunofixation" (see also claim 20) has, however, to be construed as meaning suitable for the intended use.

In that respect it is considered that the antimicrobial mouthwash composition of D1 (see col. 8, line 44 et seq.; claim 1) could not be used as a buffer within the meaning of the present invention. Thus, D1 relates to distant state of the art.

- 3) The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1 - 8 and 10 is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

Each of D2 - D7 discloses buffers comprising benzoic acid and/or a salt thereof in zone electrophoresis.

D2 mentions benzoic acid, benzoate and TRIS buffer (see Figures 1 - 4), with, e.g., benzoic acid at 30 mM and 100 mM thus being prejudicial to the novelty of claims 1, 5 - 8 and 10.

D3 (page 28, r. col. bottom paragraph), D4 (page 214, bottom paragraph), D5 (page 298, r. col., first paragraph), D6 (page 65, r. col., paragraph 3) and D7 (page 236, r. col., paragraph 3; Figures 1, 2) relate to similar subject-matter as D2. D4 (loc. cit.) discloses in addition an electrolyte solution of 10 mM sodium benzoate with 0.5 mM tetradecyltrimethylammonium bromide. D7 (loc. cit.) makes even mention of polyacrylamide coated capillaries.

- 4) As a direct consequence of the objections raised under item 3, above, also the subject-matter of claims 21 - 22 lacks novelty for analogous reasons (Art. 33(2) PCT).
- 5) Dependent claim 9 does not appear to contain any additional features which, in combination with the feature of the claim(s) to which it refers, involve an inventive step (Art. 33(3) PCT). The concentrations claimed therein would not appear to solve a technical problem in an unexpected manner and are considered to be

merely to result of an arbitrary selection amongst many concentrations possible (e.g. in view of D2; vide supra).

The applicant held inter alia that at the particular concentration claimed the performance of the buffer was excellent. However, it can be deduced from the Table in Example 3 of the present application that the allegedly unexpected effect is displayed over a rather broad concentration range. The concentrations used in at least D2 would thus appear to also display the the said effect.

- 6) The subject-matter of claim 11, and claims 12 - 20 as dependent thereon would appear to be novel and inventive in view of the known state of the art (Art. 33(2), (3) PCT).

The arguments put forward by the applicant in this respect and in response to the Written Opinion can essentially be accepted. It would appear that, although D2 makes mention of zone electrophoresis in general, the said disclosure if taken as a whole, clearly is uniquely concerned with capillary zone electrophoresis. In addition, the applicant put forward convincingly that the differences between capillary electrophoresis and zone electrophoresis, i.e. voltage applied to and dimensions of the system, impair that the two technologies cannot be directly compared; see also the following documents submitted by the applicant:

"Introduction to Capillary Electrophoresis", Beckman, page iii (1991),

*"Capillary Electrophoresis", Dale R. Baker, John Wiley & Sons, Chapter 1,
"Introduction", Section 1.2.6 "Power Supply", page 13, third paragraph, Chapter 3,
"Modes of Capillary Electrophoresis", page 53,*

"Handbook of Capillary Electrophoresis Applications", Blackie Academic & Professional (1997), page 241, second paragraph.

Thus, the skilled person, being equipped with the teachings of the closest state of the art, would have had neither motivation nor guidance to use the known buffers for zone electrophoresis and/or immunofixation.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/DK00/00350

Section VIII

The scope of claim 21 is rendered vague due to the use of the term kit in relation to one kit component only (Art. 6 PCT). Such a wording is contrary to the technical meaning of kit.

28 June 2001

Patent Application No. PCT/DK00/00350 - PCT

5 REVISED CLAIMS

1. Zone electrophoresis and/or immunofixation buffer comprising benzoic acid and/or a salt thereof.
- 10 2. Buffer according to claim 1, comprising a salt of benzoic acid.
3. Buffer according to claim 1 or 2, comprising the sodium salt, the potassium salt, the calcium salt, the
15 magnesium salt and/or the ammonium salt of benzoic acid.
4. Buffer according to any one of claims 1-3, comprising the sodium salt of benzoic acid.
- 20 5. Buffer according to any one of claims 1-4, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.
- 25 6. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L in the gel buffer.
- 30 7. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L in the gel buffer.
- 35 8. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L,

3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L in the compartment buffer.

9. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 3.5 g/L in the compartment buffer.

10. Buffer according to any one of claims 1-9, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.

11. Use of benzoic acid and/or a salt thereof as a buffer component for zone electrophoresis and/or immunofixation.

12. Use according to claim 11, wherein a salt of benzoic acid is used.

13. Use according to claim 11 or 12, wherein the salt of benzoic acid is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt.

14. Use according to any one of claims 11-13, wherein the salt of benzoic acid is the sodium salt.

15. Use according to any one of claims 11-14, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.

16. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L in the gel buffer.

17. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L in the gel buffer.
- 5 18. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L in the compartment buffer.
- 10 19. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of 3.5 g/L in the compartment buffer.
- 15 20. Use according to any one of claims 11-19, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.
- 20 21. Kit for zone electrophoresis and/or immunofixation comprising a buffer as defined in any one of claims 1-10.
- 25 22. Kit according to claim 21 further comprising gels containing the buffer as defined in any one of claims 1-10, staining solutions, antibodies, blotters, templates, and/or fixation reagents.

CLAIMS

1. Buffer for use in zone electrophoresis and/or immunofixation comprising benzoic acid and/or a salt thereof.
5
2. Buffer according to claim 1, comprising a salt of benzoic acid.
- 10 3. Buffer according to claim 1 or 2, comprising the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt of benzoic acid.
4. Buffer according to any one of claims 1-3, comprising
15 the sodium salt of benzoic acid.
5. Buffer according to any one of claims 1-4, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.
20
6. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to
25 8 g/L, or 7.5 to 8 g/L in the gel buffer.
7. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L in the gel buffer.
30
8. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L in
35 the compartment buffer.

9. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 3.5 g/L in the compartment buffer.

5

10. Buffer according to any one of claims 1-9, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.

10 11. Use of benzoic acid and/or a salt thereof as a buffer component for zone electrophoresis and/or immunofixation.

12. Use according to claim 11, wherein a salt of benzoic acid is used.

15

13. Use according to claim 11 or 12, wherein the salt of benzoic acid is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt.

20

14. Use according to any one of claims 11-13, wherein the salt of benzoic acid is the sodium salt.

25 15. Use according to any one of claims 11-14, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.

30 16. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L in the gel buffer.

35 17. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L in the gel buffer.

18. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L,
5 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L in the compartment buffer.

19. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of
10 3.5 g/L in the compartment buffer.

20. Use according to any one of claims 11-19, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.

15

21. Kit for zone electrophoresis and/or immunofixation comprising a buffer as defined in any one of claims 1-10.

22. Kit according to claim 21 further comprising gels
20 containing the buffer as defined in any one of claims 1-10, staining solutions, antibodies, blotters, templates, and/or fixation reagents.

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(54) Title: **BENZOATE BUFFERS FOR ZONE ELECTROPHORESIS AND IMMUNOFIXATION**

(57) Abstract: Buffers comprising benzoic acid and/or a salt thereof are disclosed, which buffers are suitably for use for zone electrophoresis and/or immunofixation. Furthermore, the use of benzoic acid and/or a salt thereof for zone electrophoresis and/or immunofixation is disclosed. Kits for zone electrophoresis and/or immunofixation are also provided.

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A. CLASSIFICATION OF SUBJECT MATTER

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EPO-Internal, WPI Data, CHEM ABS Data, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TARNAI, M. ET AL: "Capillary electrophoretic separation of mono- and dimaltosyl-.beta.-cyclodextrins and determination of the stability constants of their benzoate complexes." CHROMATOGRAPHIA, vol. 48, no. 5-6, 1998, pages 383-387, XP000856531 cited in the application page 384, column 1, paragraph 4; figures 1-4 --- -/--	1-22

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- *P* document published prior to the international filing date but later than the priority date claimed

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INTERNATIONAL SEARCH REPORT

Inte bnal Application No
PCT/DK 00/00350

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	<p>AHUMADA, I. ET AL: "Determination of organic acids and phosphate in soil aqueous extracts by capillary zone electrophoresis." COMMUNICATIONS IN SOIL SCIENCE AND PLANT ANALYSIS, vol. 30, no. 1-2, 1999, pages 213-220, XP000856533 cited in the application abstract</p> <p style="text-align: center;">---</p>	1-22
X	<p>ARELLANO, M. ET AL: "Capillary electrophoresis and indirect UV detection as a fast and simple analytical tool for bacterial taxonomy." J. CHROMATOGR., A (PRESENTED AT THE 9TH INTERNATIONAL SYMPOSIUM ON HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS HELD IN ANAHEIM, CA, USA, 26-30 JAN 1997), vol. 781, no. 1-2, 1997, pages 497-501, XP004094582 cited in the application figure 2</p> <p style="text-align: center;">---</p>	1-22
X	<p>LUNA, E. A. ET AL: "Evaluation of the utility of capillary electrophoresis for the analysis of sulfobutyl ether.beta.-cyclodextrin mixtures" JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS, vol. 15, no. 1, 1996, pages 63-71, XP000856460 cited in the application abstract</p> <p style="text-align: center;">---</p>	1-22
X	<p>CHANKVETADZE, BEZHAN ET AL: "Capillary electrophoresis enantioseparation of noncharged and anionic chiral compounds using anionic cyclodextrin derivatives as chiral selectors" JOURNAL OF CAPILLARY ELECTROPHORESIS, vol. 2, no. 5, 1995, pages 235-240, XP000856503 cited in the application abstract; figures 1,2</p> <p style="text-align: center;">---</p>	1-22
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 00/00350

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 891 422 A (CARLIN EDWARD ET AL) 6 April 1999 (1999-04-06) column 8, line 36 - line 36; claim 1 ---	1-10,22
A	US 4 321 119 A (AMBLER JEFFREY) 23 March 1982 (1982-03-23) cited in the application the whole document -----	1-22

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/DK 00/00350

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5891422	A	06-04-1999	NONE	
US 4321119	A	23-03-1982	AU 6189780 A EP 0034233 A JP 56076040 A	14-05-1981 26-08-1981 23-06-1981

BENZOATE BUFFERS FOR ZONE ELECTROPHORESIS AND IMMUNOFIXATION

The present invention relates to buffers comprising benzoic acid and/or a salt thereof for use in zone electrophoresis and/or immunofixation. The invention further relates to the use of benzoic acid and/or a salt thereof as buffer components for zone electrophoresis and/or immunofixation. The invention also concerns kits for zone electrophoresis and/or immunofixation. An optimised combination of the gel buffer and the compartment buffer is disclosed herein giving sharper and easier identifiable protein bands or protein pattern. A special advantage being that the buffers disclosed herein are non-hazardous.

15

BACKGROUND OF THE INVENTION

The principle of immunofixation was described by Alfonzo and Wilson in 1964 (ref. 1). The method was later modified by Alper and Johnson in 1969 and used for identification of genetic protein variants (ref. 2). Immunofixation is a widely used diagnostic method. It is a rapid, important and useful tool for the examination and identification of various protein abnormalities in serum, urine, cerebrospinal and synovial fluids.

25

The immunofixation procedure can be used for the identification of any single protein band of an electrophoresis. The technique is a combination of zone electrophoresis followed by immunofixation using monospecific antibodies. In this way it is possible to separate and identify different proteins in a biological mixture according to their physicochemical properties and antigenic properties.

35

The immunofixation procedure is most frequently used for the detection of monoclonal immunoglobulins in serum and Bence Jones proteins in urine.

5 Usually, barbital buffers comprising barbituric acid and/or sodium barbiturate are used (ref. 3). In fact, this use is recommended as barbituric acid/sodium barbiturate provide a good separation of all protein
10 are hazardous compounds which potentially cause irritation by contact with the skin, the eyes or the respiratory system, and which in extreme cases even may cause death. In more and more countries, the use of barbituric acid and sodium barbiturate in buffers is
15 therefore prohibited.

Thus, buffer components which can replace barbituric acid and sodium barbiturate and which further possess the advantages of barbituric acid and/or sodium barbiturate
20 are still needed. The present invention provides such compounds which can replace barbituric acid and sodium barbiturate.

SUMMARY OF THE INVENTION

25

In a first aspect, the present invention relates to buffers comprising benzoic acid and/or a salt thereof for zone electrophoresis and/or immunofixation.

30 In another aspect, the present invention relates to the use of benzoic acid and/or a salt thereof as buffer components for zone electrophoresis and/or immunofixation.

In a third aspect, the present invention concerns kits for zone electrophoresis and/or immunofixation comprising a buffer as described herein.

5 The present invention is described in detail in the following.

BRIEF DESCRIPTION OF THE FIGURE

10 Figure 1 shows an immunofixation gel obtained following the procedures described in Example 1.

Figures 2A, 2B, 2C and 2D show four patient serum samples subjected to zone electrophoresis and immunofixation with
15 the buffer of the invention comprising the sodium salt of benzoic acid.

Figures 3A, 3B, 3C and 3D show the four patient serum samples as shown in Figures 2A, 2B, 2C and 2D subjected
20 to zone electrophoresis and immunofixation with the buffer of the invention comprising the ammonium salt of benzoic acid.

DETAILED DESCRIPTION OF THE INVENTION

25

The present invention relates buffers for use in zone electrophoresis and/or immunofixation comprising benzoic acid and/or a salt thereof.

30 From Chromatographia Vol. 48, No. 5/6, 383-387 (1998) (ref. 4), Deutsche Lebensmittel-Rundschau 94. Jahrg., Heft 1, 28-30 (1998) (ref. 5), Commun. Soil Sci. Plant Anal. 30 (1 & 2), 213-220 (1999) (ref. 6), Journal of Chromatography A. 781, 497-501 (1997) (ref. 7), Journal
35 of Pharmaceutical and Biomedical Analysis 15, 63-71 (1996) (ref. 8), and J. Cap. Elec. Vol. 2, No. 5, 235-240

(1995) (ref. 9) buffers containing benzoic acid or sodium benzoate are known. The buffers are used in capillary electrophoresis for the determination of i.a. organic acids, phosphate, phytin acid, short-chain fatty acids, and cyclodextrins. The principle of capillary electrophoresis is very different from the principles of zone electrophoresis in gels and immunofixation.

From US 4,321,119 (ref. 10), a non-barbiturate buffer composition for use in the electrophoretic separation of proteins into fractions is known, said buffer comprising a water soluble salicylate such as sodium salicylate and an inorganic salt. The salicylate is used in a concentration of from 0.5 to 1 g salicylate to 1.5 to 3 g inorganic salt.

In particular, the buffer of the invention may comprise a salt of benzoic acid. Examples of suitable salts is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt of benzoic acid. The buffer of the invention may comprise one or more of these salts of benzoic acid optionally in combination with benzoic acid itself. Accordingly, benzoic acid or one or more salts of benzoic acid may be used alone, or benzoic acid and one or more salts of benzoic acid may be used in combination. In a preferred embodiment, the buffer of the invention comprises a salt of benzoic acid, in particular the sodium salt of benzoic acid.

The buffer of the invention is particularly suitable for separation of proteins, serum proteins, and immunoglobulins in zone electrophoresis and/or immunofixation. Proteins are large molecules which are susceptible to denaturation, thus, making them sensitive to the conditions employed in the separation procedure. E.g. heat development during the electrophoresis

procedure may result in destruction of the protein structure. Increased ionic strength of the running buffer increases the electric conductivity, thus leading to an increased heat development. This can be avoided e.g. by using heavy cooling procedures during the electrophoresis procedure. Alternatively, low concentration of ion-providing components of the running buffer can be used. For instance, in US 4,321,119, a relatively low concentration of salicylate is used.

However, it has surprisingly been found that a buffer comprising benzoic acid and/or a salt thereof does not suffer from the above drawbacks. The benzoic acid and the salts thereof can be used in relatively high concentrations without the need for additional cooling in order to avoid breakdown of the protein structure or denaturation. An additional advantageous feature of the buffer is that the benzoic acid and the salts thereof in general act as preserving agents. This is especially observed in the case of sodium benzoate (the sodium salt of benzoic acid).

The buffer of the invention is used as a gel buffer and/or as a compartment buffer. The term "buffer" includes both. The gel buffer and the compartment buffer may have the same acid/salt concentration, or the concentrations of the gel buffer and the compartment buffer may be different.

The gel buffer suitably comprises benzoic acid and/or a salt thereof in a concentration of from 1 to 10 g/L. In a preferred embodiment, the buffer comprises benzoic acid and/or a salt thereof in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L. In particular, the concentration of benzoic

acid and/or the salt thereof may be 7 g/L. Surprisingly, it seems as if the concentration range yielding a good separation is peak-like, having a maximum in the concentration range from 6.5 to 7.5 g/L, more specifically around a concentration of about 7 g/L.

The compartment buffer suitably comprises benzoic acid and/or a salt thereof in a concentration of from 1 to 10 g/L. In a preferred embodiment, the buffer comprises benzoic acid and/or a salt thereof in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L. In particular, the concentration of benzoic acid and/or the salt thereof may be 3.5 g/L. Surprisingly, it seems as if the concentration range yielding a good separation is peak-like, having a maximum in the concentration range 3 to 4 g/L, more specifically around a concentration of about 3.5 g/L.

It lies within the scope of the present invention to use each of the components (benzoic acid itself or any of its salts) in the concentrations specified above.

The zone electrophoresis/immunofixation procedure is a very powerful tool in the early diagnosis of various diseases. Frequently, the disease can be diagnosed even before the patient experiences symptoms of the disease. Therefore, a distinct separation of the protein bands is of crucial importance in order to make a reliable diagnosis. Especially the separation of the immunoglobulins is of major importance. These proteins are produced in the bone marrow and their appearance in immunofixation reflects the status of the bone marrow. A cancer disease in the bone marrow affecting the plasma cells may lead to changed synthesis of the immunoglobulins and thereby the appearance of these

immunoglobulins in immunofixation. The normal heterogeneous pattern is most frequently changed into a pattern of distinct bands with different mobility. In particular in such cancers an early treatment regime is important for the survival of the patient and also the treatment, e.g. chemotherapy, radiation and/or plasma-pheresis, as such influences the patient's well-being to a major extent. As evident from the Figures, the buffers of the invention provide such good and distinct separation enabling a reliable diagnosis.

The buffer of the invention may further comprise additional components such as Tris and/or Tricine and/or calcium lactate and/or sodium azide.

15

The benzoic acid compounds as defined above are suitable as buffer components, in particular as gel buffers and compartment buffers. They are much less hazardous than the conventionally used barbiturates. Furthermore, no hazard labelling of the buffers is required. It has further been shown (cf. Example 1) that the buffers of the invention seem to provide sharper and more well defined bands than the conventionally used barbiturate-containing buffers. Also, the use of a compartment buffer having a lower salt/acid concentration than the gel buffer may be advantageous. This may inhibit the heat development during electrophoresis and yield sharper and more well defined bands.

Furthermore, the buffer may contain one or more additional components such as buffering agents, preserving agents, colouring agents, salts, detergent and surfactants.

In a special embodiment of the buffer of the present invention, the gel buffer comprises the sodium salt of

benzoic acid in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L. In a preferred embodiment, the concentration of the sodium salt of benzoic acid is from 6.5 to 7.5 g/L, in particular 7 g/L.

In a special embodiment of the buffer of the present invention, the compartment buffer comprises the sodium salt of benzoic acid in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L. In a preferred embodiment, the concentration of the sodium salt of benzoic acid is from 3 to 4 g/L, in particular 3.5 g/L.

In particular, the gel buffer may have a salt concentration of 7 g/L, and the compartment buffer a concentration of 3.5 g/L.

As mentioned, the buffer is for use in zone electrophoresis, and/or immunofixation. The test samples are suitably serum, urine, cerebrospinal or synovial fluids.

In another aspect, the present invention relates to the use of benzoic acid and/or a salt thereof as a buffer component for zone electrophoresis and/or immunofixation.

Benzoic acid or a salt of benzoic acid may be used alone, or benzoic acid and one or more salts of benzoic acid may be used in combination. Examples of suitable salts of benzoic acid are the sodium salt, the potassium salt, the calcium salt, the magnesium salt and the ammonium salt. In a preferred embodiment, the salt of benzoic acid is the sodium salt.

As mentioned above, it has surprisingly been found that benzoic acid or the salts thereof can be used in relatively high concentrations. Thus, in one embodiment, the present invention relates to the use of benzoic acid and/or a salt thereof, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L. In particular, in the gel buffer, benzoic acid and/or the salt thereof may be used in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L. In particular the concentration may be from 6.5 to 7.5 g/L, like 7 g/L. In particular, in the compartment buffer, benzoic acid and/or the salt thereof may be used in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L. In particular the concentration may be from 3 to 4 g/L, like 3.5 g/L.

In a particular embodiment, the sodium salt of benzoic acid in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L is used as a component of the gel buffer. In a preferred embodiment, the sodium salt of benzoic acid in a concentration of from 6.5 to 7.5 g/L, in particular a concentration of 7 g/L, is used as a component of the gel buffer.

In a particular embodiment, the sodium salt of benzoic acid in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L is used as a component of the compartment buffer. In a preferred embodiment, the sodium salt of benzoic acid in a concentration of from 3 to 4 g/L, in particular a concentration of 3.5 g/L, is used as a component of the compartment buffer.

In a further embodiment, the present invention relates to the use of benzoic acid and/or a salt thereof in a buffer for electrophoresis and/or immunofixation, wherein the
5 buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.

In third aspect, the present invention relates to kits for zone electrophoresis and/or immunofixation, which
10 kits comprise a buffer as defined above.

In one embodiment, the kit further comprises gels containing the buffer of the invention, staining solutions, antibodies (e.g. rabbit immunoglobulins),
15 blotters, templates, fixation and/or reagents.

The gel to be used is suitably an agarose gel. The agarose gel may suitably be provided in a ready-to-use packing containing the buffer of the invention. The
20 buffer of the invention may thus be used as compartment buffer as well as gel buffer for supporting medias such as agarose, starch, polyacrylamide etc.

The invention is further illustrated by the following, non-limiting example.
25

EXAMPLES

EXAMPLE 1

30

Materials. Agarose Gel, 10 plates (gel buffer). Ready-to-use. Each plate is 8.3x10.2 cm and contains on a transparent, flexible plastic backing, agarose gel containing the buffer of the invention comprising sodium
35 benzoate (i.e. sodium salt of benzoic acid) or ammonium

benzoate (i.e. the ammonium salt of benzoic acid) (1% gel, 99% buffer) preserved with sodium azide.

5 Concentrated Buffer. 3x75 mL (13.33 x concentrated) buffer of the invention preserved with sodium azide. The content of each of the bottles of buffer is diluted prior to use to a total volume of 1000 mL with distilled water. The diluted buffer contains sodium benzoate (3.5 g/L) or
10 ammonium benzoate (3.5 g/L), Tris (3.6 g/L), Tricine (0.6 g/L), calcium lactate (0.75 g/L), and sodium azide (0.04 g/L).

Concentrated Staining Solution. 75 mL (4 x concentrated). Amido Black in 5% acetic acid. The Staining Solution is
15 diluted prior to use to a total volume of 300 mL with distilled water. The concentration of Amido Black in the diluted Solution is 5 g/L.

Test Sample. Serum samples, optionally freshly drawn. 33
20 samples were tested.

Sample Template. 10 pieces.

Antibody Template. 10 pieces.
25

Gel Blotter. Pre-cut disposable, filter paper, 1 package, 40 sheets.

Sample Blotter. Pre-cut disposable, filter paper, 1
30 package, 10 sheets.

Drying Blotter. Pre-cut disposable filter paper, 2 package, 20 sheets each.

Fixation Reagents. Protein Fixative Solution 1.0 mL containing 7% sulphosalicylic acid and 5% acetic acid. Green dyed.

5 Rabbit Anti-Human IgG. Specific for γ -chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

10 Rabbit Anti-Human IgA. Specific for α -chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

15 Rabbit Anti-Human IgM. Specific for μ -chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

20 Rabbit Anti-Human Kappa Light Chains. Specific for kappa light chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

Rabbit Anti-Human Lambda Light Chains. Specific for lambda light chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

25 Other reagents. Saline Solution (0.9% NaCl). For dilution of the samples and washing of the gel. Destaining Solution (acetic acid, 5%). Distilled or deionised water.

30 Equipment. Power supply 120 V constant. Electrophoresis apparatus for Agarose Gels (DAKO Electrophoresis Apparatus Code No. S 2200). Pipettes (5 μ L, 80 μ L). Containers for washing, staining and destaining of Agarose Gels (DAKO Washing and Staining Accessory Kit Code No. S 2201). Glass plate (minimum 11x11 cm) plus a
35 weight of approximately 1 kg for pressing the gel. Hair dryer or a drying oven (maximum 90°C).

Additional Reagents. Rabbit Anti-Human IgD (DAKO Code No. A 0093), specific for δ -chains, immunoglobulin fraction, preserved with 15 mM sodium azide. Rabbit Anti-Human IgE (DAKO Code No. A 0094), specific for ϵ -chains, immunoglobulin fraction, preserved with 15 mM sodium azide. Rabbit Anti-Human Kappa Free Light Chains (DAKO Code No. A 0100), specific for kappa free light chains, immunoglobulin fraction, preserved with 15 mM sodium azide. Rabbit Anti-Human Lambda Free Light Chains (DAKO Code No. A 0101), specific for lambda free light chains, immunoglobulin fraction, preserved with 15 mM sodium azide.

Preparation of specimens. All serum specimens should preferably be diluted with saline solution just prior to use. For the reference pattern, serum should be diluted 1:4 (1 part serum + 3 parts Saline Solution). For the immunofixation patterns serum should be diluted 1:15 (1 part serum + 14 parts saline solution). For serum suspected of containing low levels of monoclonal immunoglobulins, a dilution of 1:4 is recommended. For serum specimens suspected of containing high levels of monoclonal immunoglobulin (>30 g/L), a dilution of 1:31 may be suitable.

For the detection of Bence Jones proteins in urine, the urine sample should be concentrated (e.g. by ultrafiltration) to a total protein concentration of at least 1 g/L. This concentrated urine is applied in all slots. The light chain antibodies as described above will precipitate kappa or lambda chains whether they are free or still part of the immunoglobulin molecule. In order to determine if detected light chains are present as free light chains in the urine, special antibodies as

described above against free kappa and free lambda light chains could be employed.

Assay procedure

5 Zone Electrophoresis (separation of the proteins). All samples are prepared as described above. The Agarose Gel is removed from the foil package and placed on a level surface. Excess moisture is removed from the gel surface by gentle blotting with a Gel Blotter. The Sample
10 Template is placed on the surface of the gel so that the slots are in alignment with the arrows located on the edges of the gel. 5 μ L of the pre-diluted serum sample is applied across each slot. The 1:4 serum dilution is applied in the slot marked Ref., and the 1:15 serum
15 dilution in the other 5 slots. The sample is allowed to diffuse into the gel for 5 minutes, and then the sample template is blotted gently with a Sample Blotter in order to remove excess sample liquid. The Blotter is discarded, and the Sample Template is carefully removed and
20 discarded.

Electrophoresis. The DAKO Electrophoresis Apparatus is filled with 300 mL diluted buffer (150 mL in each compartment). The gel is placed in the apparatus so as to
25 form an arch (gel side down) in such a way that the (-) side of the gel dips into the cathode compartment (-), and that the (+) side of the gel dips into the anode compartment (+). The lid is placed on the apparatus and power supply is connected. The voltage is set to 120 V
30 and the electrophoresis is continued for 25 minutes. Upon completion of the electrophoresis, the power supply is disconnected, and the gel is carefully removed from the apparatus and placed on a level surface, gel side up. The electrophoresis buffer is discarded.

Immunofixation (specific precipitation of the separated proteins). The surface of the gel is gently blotted with a Gel Blotter. The Gel Blotter is removed immediately and discarded. The Antibody Template is placed over the surface of the gel so that the troughs of the Template are in alignment with those printed on the plastic backing of the gel. It should be ensured that a close contact between the Template and the surface of the gel is obtained. The Template is gently rubbed in order to remove air bubbles. The following is applied: Ref.: 80 µL of Protein Fixative Solution, IgG: 80 µL of Anti-IgG, IgA: 80 µL of Anti-IgA, IgM: 80 µL of Anti-IgM, K: 80 µL of Anti-Kappa, and L: 80 µL of Anti-Lambda. It should be ensured that the volume is evenly distributed within the trough. Furthermore, the surface of the gel should not be touched. The gel is incubated with the Antibody Template in a humid box for 15 minutes at room temperature. Subsequently, the gel is placed on a levelled surface and the Antibody Template is carefully removed.

Pressing, washing, staining, destaining and drying (removal of non-precipitated proteins and staining of the precipitated protein bands). The containers of the DAKO Washing and Staining Accessory Kit are filled in the following way: Washing: 1 container with 300 mL Saline Solution. Staining: 1 container with 300 mL diluted Staining Solution. Rinsing: 1 container with 300 mL distilled water. Destaining: 1 container with 300 mL acetic acid, 5%. The gel is covered with one sheet of Gel Blotter, two sheets of Drying Blotter and a glass plate. The gel is pressed under a weight of approximately 1 kg for 10 minutes. Subsequently, the Blotters are removed and discarded, and the gel is immersed in saline solution and washed for 10 minutes without agitation. The pressing procedure is repeated as previously described. After pressing, the Blotters are discarded, and the gel is

dried in a current of hot air or, alternatively, dried in a drying oven (maximum temperature 90°C) for approximately 5 minutes.

- 5 Staining is performed for 5 minutes in the Diluted Staining Solution.

Excess Staining Solution is rinsed off in distilled water before destaining. Destaining is performed in fresh
10 Destaining Solution for approximately 2 minutes, or until the background has a faint blue colour.

Finally, the gel is dried for 5 minutes as previously described or until the gel is completely dry.

15

In Figures 1, 2 (A, B, C and D) and 3 (A, B, C and D) the results obtained are shown. Each immunofixation gel shows the results of one patient serum sample. The lane at the left is a reference lane showing all serum proteins in
20 the serum sample in the order albumin (top), followed by alpha, beta, and gamma globulins (immunoglobulins). The following lanes are a visualisation of the patient's immunoglobulins established by the use of specific antibodies. From left to right, the gel shows IgG, IgA,
25 IgM, kappa light chain, and lambda light chain.

The results shown in Figure 1 are obtained using the sodium benzoate buffer.

- 30 The results shown in Figure 2 are obtained using the sodium benzoate buffer. Figure 2A shows a patient having clearly defined double bands in the gamma region. The bands can be identified as IgM, kappa. Figure 2B shows a patient having strong double bands in the gamma region.
35 The bands are stronger than in Figure 2A. The bands can be identified as IgM, kappa. Figure 2C shows a patient

having a strong band which can be identified as IgA, lambda. Figure 2D shows a patient having a very strong band which can be identified as IgG, lambda.

5 The results shown in Figure 3 are obtained using the ammonium benzoate buffer. Figures 3A, 3B, 3C and 3D shows the serum samples as in Figures 2A, 2B, 2C and 2D. The only difference being that the buffer used is an ammonium benzoate buffer. As appears from the figure, the results
10 obtained using the ammonium benzoate buffer are not as excellent as the results obtained using the sodium benzoate buffer. However, this might be due to lack of optimisation.

15 Results. The overall function of the buffer is comparable to conventional barbiturate-containing buffer (DAKO Code No. K 0390). Furthermore, sharper and more well defined bands were obtained. A further advantage of the novel buffer is that it is non-hazardous.

20

EXAMPLE 2

A series of zone electrophoresis and immunofixation procedures using the electrophoresis/immunofixation set-
25 up described in Example 1 were performed. The buffer used for this experiment corresponded with regard to the components to the sodium benzoate buffer described in Example 1, however, the gel buffer content of sodium benzoate was varied between 1 and 10 g/L, and the
30 compartment buffer content of sodium benzoate was varied accordingly. The separation of the proteins was evaluated and rated 3 (excellent separation), 2 (acceptable separation) or 1 (bad separation). The results obtained are shown in the table below.

35

g/L sodium benzoate in gel buffer	Performance
1	1
2	1
3	1
4	2
5	2
7	3
10	1

As it appears from the table, it seems as if the performance of sodium benzoate is peak-like around a concentration maximum of about 7 g/L. Of course this peak may be shifted depending on the nature and characteristics of the other components of the buffer.

EXAMPLE 3

10

A series of zone electrophoresis and immunofixation procedures using the electrophoresis/immunofixation set-up described in Example 1 were performed. The buffer used for this experiment corresponded with regard to the components to the sodium benzoate buffer described in Example 1, however, the gel buffer content of sodium benzoate being 7 g/L, whereas the compartment buffer content of sodium benzoate was varied between 0.8 and 7 g/L. The separation of the proteins was evaluated and rated 3 (excellent separation), 2 (acceptable separation) or 1 (bad separation). The results obtained are shown in the table below.

g/L sodium benzoate in compartment buffer	Performance
0.8	1
1.2	1
1.8	2
3.5	3
4.7	3
7	2

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CLAIMS

1. Buffer for use in zone electrophoresis and/or immunofixation comprising benzoic acid and/or a salt thereof.
5
2. Buffer according to claim 1, comprising a salt of benzoic acid.
- 10 3. Buffer according to claim 1 or 2, comprising the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt of benzoic acid.
4. Buffer according to any one of claims 1-3, comprising
15 the sodium salt of benzoic acid.
5. Buffer according to any one of claims 1-4, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.
20
6. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to
25 8 g/L, or 7.5 to 8 g/L in the gel buffer.
7. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L in the gel buffer.
30
8. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L in
35 the compartment buffer.

9. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 3.5 g/L in the compartment buffer.

5

10. Buffer according to any one of claims 1-9, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.

10 11. Use of benzoic acid and/or a salt thereof as a buffer component for zone electrophoresis and/or immunofixation.

12. Use according to claim 11, wherein a salt of benzoic acid is used.

15

13. Use according to claim 11 or 12, wherein the salt of benzoic acid is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt.

20

14. Use according to any one of claims 11-13, wherein the salt of benzoic acid is the sodium salt.

15. Use according to any one of claims 11-14, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.

16. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L in the gel buffer.

17. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L in the gel buffer.

18. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L in the compartment buffer.

19. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of 3.5 g/L in the compartment buffer.

20. Use according to any one of claims 11-19, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.

15

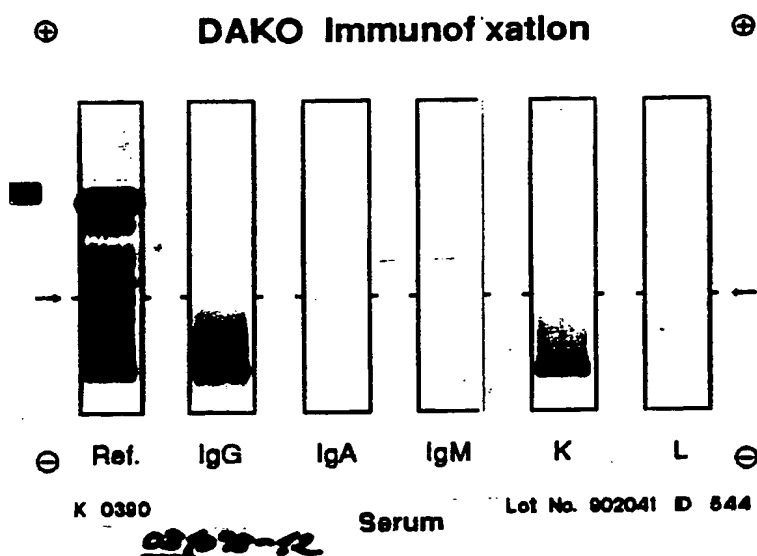
21. Kit for zone electrophoresis and/or immunofixation comprising a buffer as defined in any one of claims 1-10.

22. Kit according to claim 21 further comprising gels containing the buffer as defined in any one of claims 1-10, staining solutions, antibodies, blotters, templates, and/or fixation reagents.

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Fig. 1



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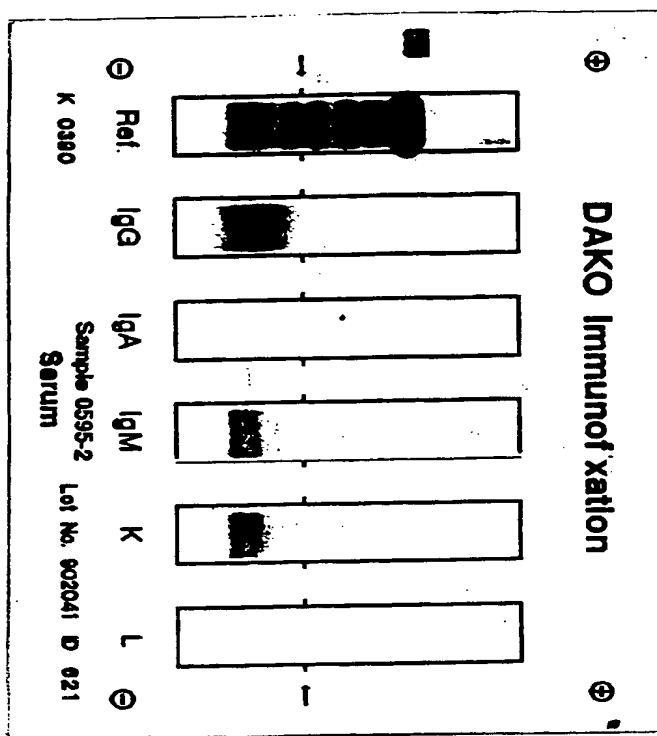


Fig. 2A

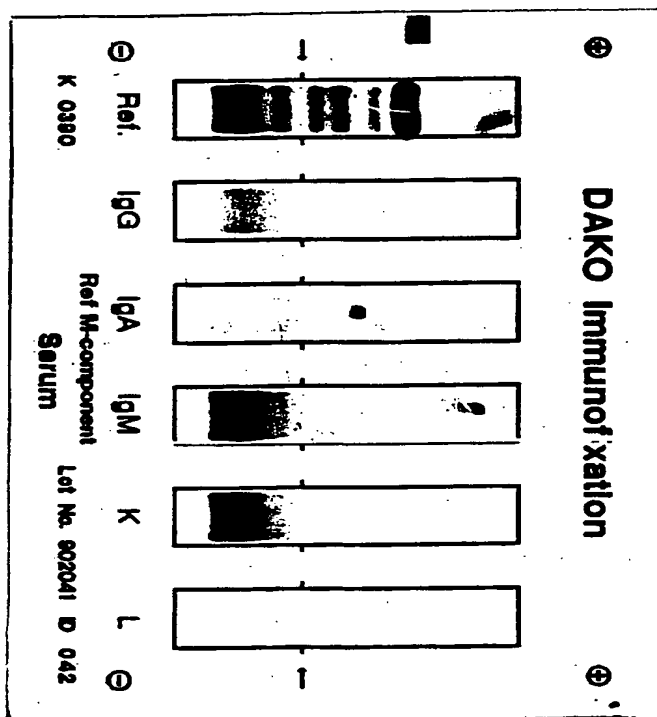
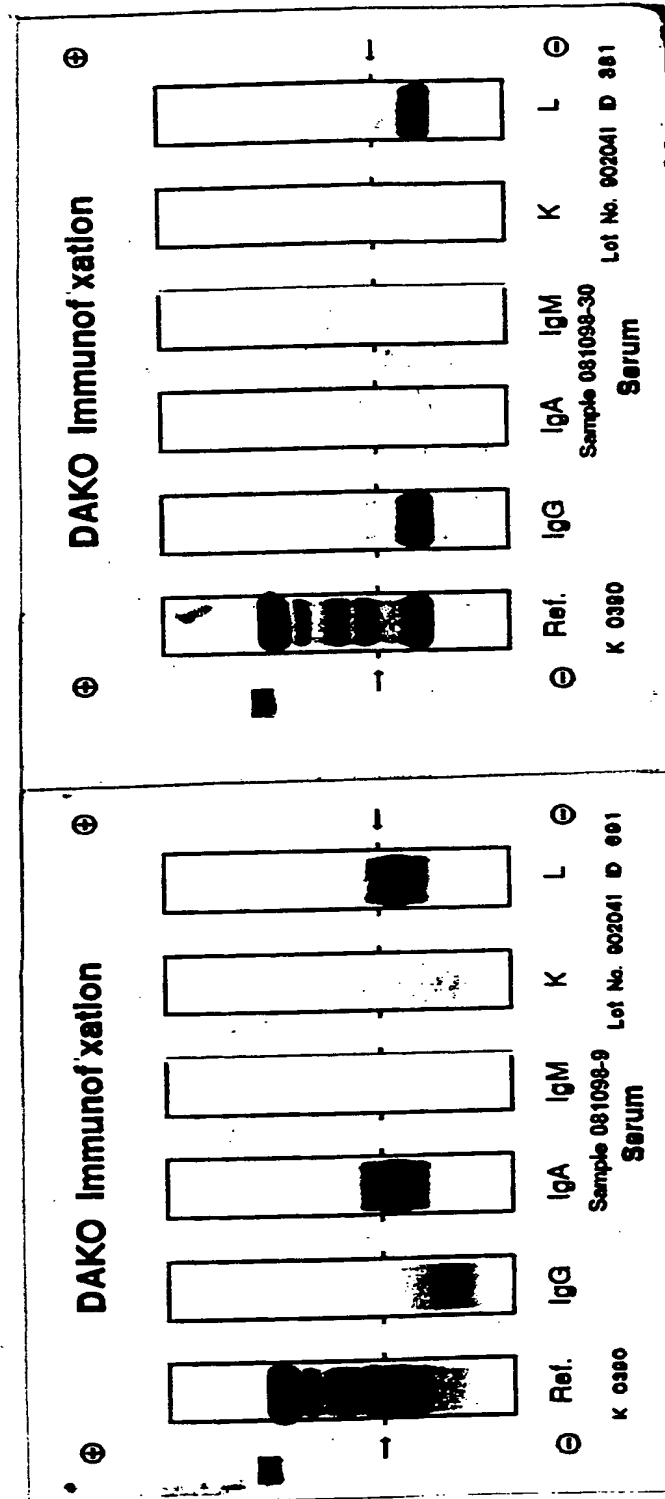


Fig. 2B

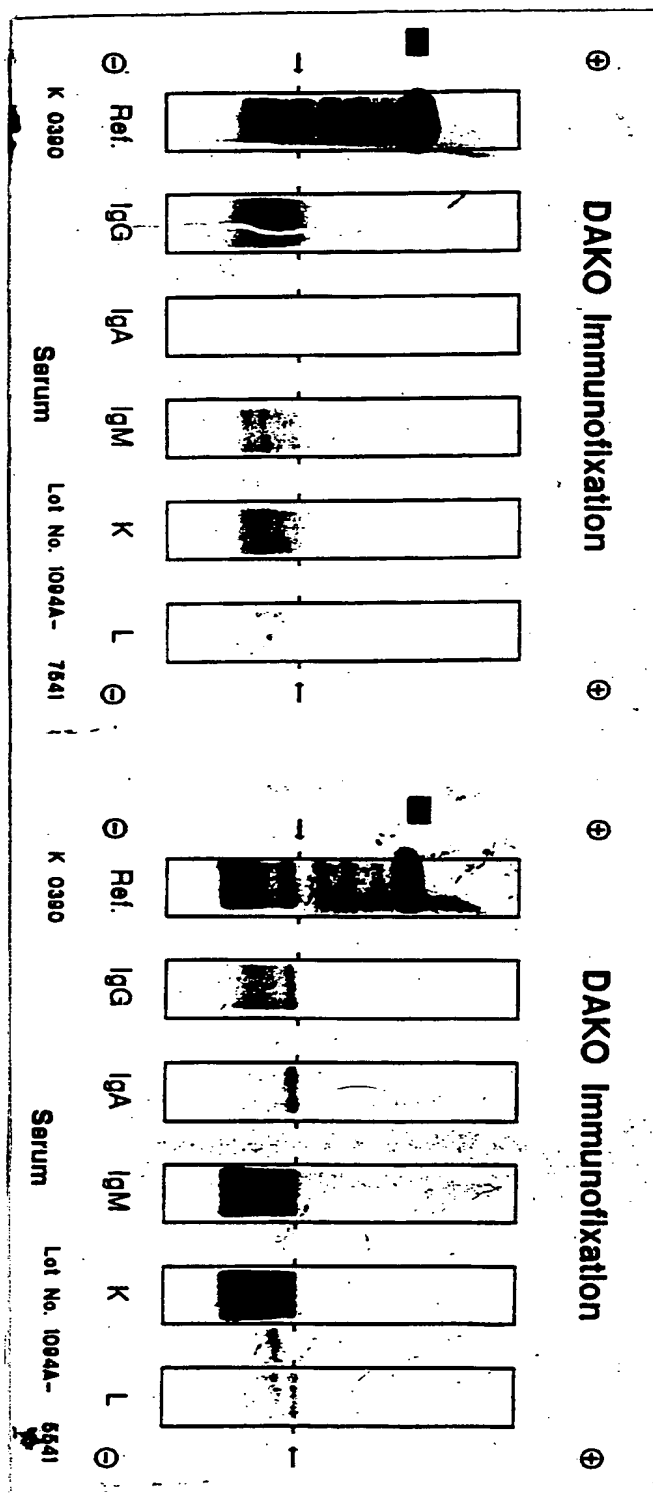
Fig. 2D



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Fig. 3A

Fig. 3B



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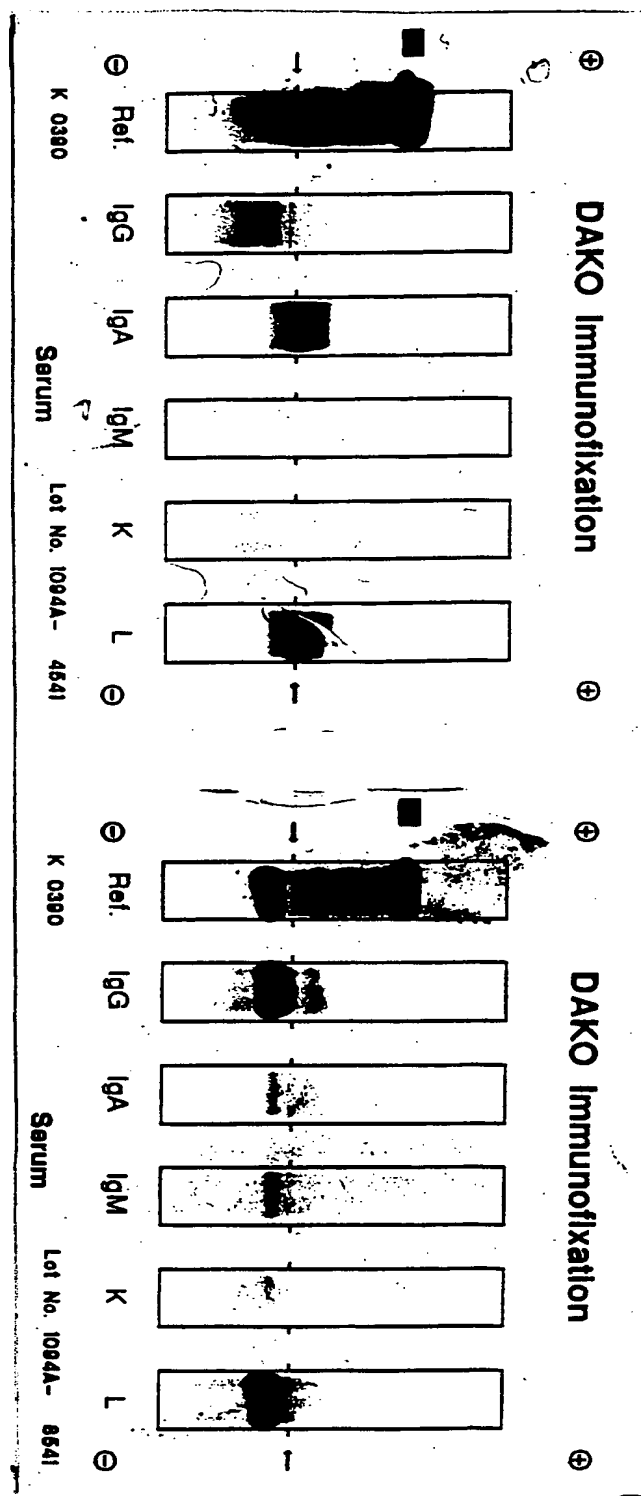


Fig. 3C

Fig. 3D